

Applicant : M. Amin Armaout et al.
Serial No. : 09/758,493
Filed : January 11, 2001
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Attorney's Docket No.: 00786-804001 / MGH 1721.1



REMARKS

Applicants hereby submit that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825. The amendments in the specification merely insert the paper copy of the Sequence Listing and sequence identifiers in the specification. No new matter has been added.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment.

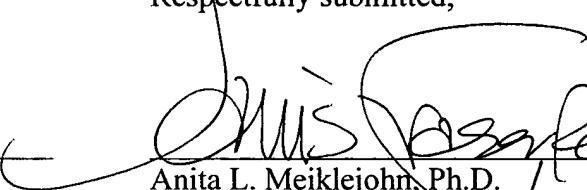
Please apply any charges or credits to Deposit Account No. 06-1050.

Date

Aug. 8, 2001

Respectfully submitted,

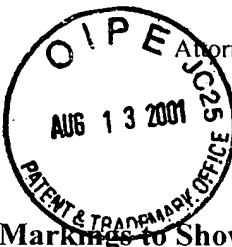
Anita L. Meiklejohn, Ph.D.
Reg. No. 35,283


Reg. No. 34819

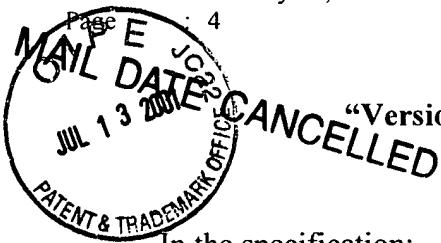
Fish & Richardson P.C.
225 Franklin Street
Boston, MA 02110-2804
Telephone: (617) 542-5070
Facsimile: (617) 542-8906

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“Version With Markings to Show Changes Made”



In the specification:

Paragraph beginning at page 6, line 22, has been amended as follows:

Figure 5 depicts an alignment of the A domains of nine alpha integrin α subunit (CD11b (SEQ ID NO:1), CD11c (SEQ ID NO:2), CD11d (SEQ ID NO:3), CD11a (SEQ ID NO:4), alpha 1 (SEQ ID NO:5), alpha 2 (SEQ ID NO:6), alpha 10 (SEQ ID NO:7), alpha 11 (SEQ ID NO:8), and alpha E (SEQ ID NO:9)). In this alignment, the invariant Ile (I316) is indicated by an arrow.

Paragraph beginning at page 6, line 28, has been amended as follows:

Figure 7 is an alignment of the A-like domains of eight integrin β subunits (b3 (SEQ ID NO:10), b5 (SEQ ID NO:11), b6 (SEQ ID NO:12), b1 (SEQ ID NO:13), b2 (SEQ ID NO:14), b7 (SEQ ID NO:15), b8 (SEQ ID NO:16), and b4 (SEQ ID NO:17)). In this alignment, the residue corresponding to the invariant Ile in β subunits is indicated by an arrow.

Paragraph beginning at page 7, line 13, has been amended as follows:

The variant polypeptides were created using standard recombinant techniques.

Restriction and modification enzymes were purchased from New England Biolabs, Inc. (Beverly, MA), Boehringer Mannheim (Germany), or GIBCO BRL (Gaithersburg, MD). Site-directed mutagenesis was carried out in pGEX-4T-1 vector as described (Rieu et al. 1996 *J Biol Chem* 271:15858). The following mutagenic primers were used. IFAdel Fwd: 5'-TATAGGATCCGAGGCCCTCCGAGGGAGTCCTCAAGAGGGATAG-3' (SEQ ID NO:18); Reverse: 5'-CTACTCGAGTTACTTCTCCGAAGCTGGTTCTGAATGGTC-3' (SEQ ID NO:19); I-G reverse: 5'- CTACTCGAGTTAACCTCGATCGCAAAGCCCTCTC-3' (SEQ ID NO:20). Introduction of the respective mutation was confirmed by direct DNA sequencing. The Pvul-BspEI-restricted cDNA fragment of the A-domain containing the mutation was subcloned into the Pvul-BspEI-restricted CD11b cDNA, cloned into pcDNA3 plasmid, which containing full-length human CD11b (Rieu et al. 1996 *J Biol Chem* 271:15858). 11b A¹²³⁻³²¹ and

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11bA¹²³⁻³¹⁵ and 11bA^{1→G} A-domains were expressed as GST fusion proteins in *Escherichia coli* (Michishita et al. 1993 *Cell* 72:857), cleaved with thrombin and purified as described Li et al. 1999 *J. Cell Biol* 143:1523. C¹²⁹ was replaced by S in all the expressed GST-A-domain fusion form to prevent formation of disulfide-linked dimmers in solution after thrombin cleavage (not shown). Purity was confirmed by SDS-PAGE analysis.